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The Synthesis of Unnatural Morphine Derivatives as Glial Activation Inhibitors and The Synthesis of Corticosterone-Glucuronide

Morin Mae Frick
morinm.frick@gmail.com

The Synthesis of Unnatural Morphine Derivatives as Glial Activation Inhibitors

And

The Synthesis of Corticosterone-Glucuronide

by

Morin M. Frick

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And
The Synthesis of Corticosterone-Glucuronide
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Dr. Tarek Sammakia

Dr. Xiang Wang

Date:_____

The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline

Abstract:

Frick, Morin Mae (M.S., Chemistry)

The Synthesis of Unnatural Morphine Derivatives as Glial Activation Inhibitors and The Synthesis of Corticosterone-Glucuornide

Thesis directed by Professor Tarek H. Sammakia

A novel, non-neuronal mechanism to explain the development of opioid tolerance and dependance has been described. This mechanism involves the activation of spinal cord glia. Glia are important contributors to the creation of enhanced pain states via the release of neuroexcitatory and proinflammatory substances. Data suggest that glia also release neuroexcitatory substances in response to morphine, thereby opposing its effects. Inhibiting glial activation could therefore increase the clinical utility of analgesic drugs. The synthesis of advanced intermediates for the development of a series of novel morphinan-like structures that are potential glial activation inhibitors will be discussed. A Grewe-type cyclization of N-formyl compounds was the key reaction in two synthetic sequences to produce these intermediates in racemic form.

Morphine-3-glucuronide (M3G) is a major morphine metabolite detected in the cerebrospinal fluid of humans receiving systemic morphine. M3G is known to induce pain by an unknown mechanism. It has little to no affinity for opioid receptors and its pain-enhancing

effects have been proposed to significantly and progressively oppose morphine analgesia. Recent work has shown it does so via transmembrane receptors on glial cells. It has been known for some time that corticosterone (the rat version of human adrenal cortisol) displays an intense immunosuppressive effect at the time of delivery; however, recent work has shown that it produces dramatic glial and immune activation and a pro-inflammatory response later in time. Corticoserone is glucuronidated in its metabolic pathway. Corticosterone-glucuronide (CortG) was synthesized to test whether the glucuronidation is the cause of the eventual pro-inflammatory effects of corticosterone. CortG was prepared by a direct coupling of a glucuronic acid imidate donor and corticosterone in the presence of borontrifuoride etherate.

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CHAPTER ONE:

THE SYNTHESIS OF UNNATURAL MORPHINE DERIVATIVES AS GLIAL ACTIVATION INHIBITORS

Chronic pain affects more than 70 million Americans, which makes it more widespread than heart disease, cancer, and diabetes combined.[1] Unfortunately, it frequently is inadequately treated leading to enormous social costs in the form of lost productivity, needless suffering, and excessive healthcare expenditures.[1] Because pain is regarded as a significant public health issue, the search for new strategies to treat pain, acute or chronic, continues to grow. Today, opioid pharmacotherapies constitute a significant component of many pain management plans.[1] While opioids are potent analgesics, they have severe side effects including addiction, tolerance and abuse. The abuse of and addiction to opioid analgesics is a major issue that leaves doctors and patients reluctant to treat pain with these agents to the fullest extent.[2, 3]

Important areas of research that are needed to help with the problem of opioid analgesic abuse include the identification of clinical practices that minimize the risks of addiction and mistreatment. New understanding of the mechanism of pain sensation and the mechanism of action of opioid analgesics is providing new approaches to pain management. Historically, researchers have focused primarily on neuronal mechanisms of pain and analgesia while ignoring other potentially powerful modulators of nociception. Further, it has been

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[1] Hay, J., White, J., Bocher, F., Somogyi, A., Semple, T., Rounsefell, B. *The Journal of Pain.* **2009**, *3*, 316 – 322.

[2] Compton, W. M., Volkow, N. D. *Drug Alcohol Depend.* **2006**, *81*, 103.

[3] Manchikanti, L. *Pain Physician* **2006**, *9*, 287.

believed that both the detrimental and beneficial actions of opioids are mediated via potentially inseparable mechanisms reliant only on neuronal opioid receptors.[4]

Recent experimental data has implicated glia (astrocytes and microglia) as important mediators of pain. For many years glial cells were overlooked in pain research because, traditionally, they were viewed only as a support system for neurons and as having an important role in maintaining central nervous system homeostasis.[4] Today, however, glia are known to have a well-established role in initiating and maintaining increased pain states in response to peripheral nerve injury. More recently, several laboratories have documented that glia can powerfully modulate the analgesic actions of chronically administered opioids. [5,6,7,8] Based on such studies, the pharmacological targeting of glia, rather than solely neurons, may provide more effective pain control and enhanced efficacy of opioids.

The earliest evidence of the role of glial cells in pain regulation was an associative link between glial activation and neuropathic pain published in 1994.[9] This study reported the interesting observation that drugs which blocked neuropathic pain also decreased glial activation. More recently, it has been reported that when glial cells are activated, they produce and release a variety of neuroexcitatory substances including reactive oxygen species,

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[4] Watkins, L., Hutchinson, M. *The Scientific World Journal.* **2007**, *7*, 1 – 14.

[5] Johnston, I., Milligan, E., Weiseler-Frank, M., Zapata, V., Vampisi, J., Lnger, S. Martin, D., Green, P., Fleshner, M., Leinwant, L., mier, S., Watkins, L. *J. Neurosci*. **2004**, *24*, 7353 - 7365.

[6] Raghavendra, V., Tanga, F., DeLeo, J. *Neuropsychopharmacology*. **2004**, *29*, 327 – 334.

[7] Raghavendra, V., Tanga, F., Rutkowski, M., DeLeo, J. *Pain*. **2003**, *104*, 655 – 664.

[8] Song, P., Zhao, Z. *Neurosci. Res*. **2001**, *39*, 281 – 286.

[9] Garrison, C. J., Dougherty, P., Carlton, S.. *Exp. Neurol.* **1994**, *129*, 237.

[10] Watkins, L. R., Milligan, E. D., Maier, S. F. *Adv. Exp. Med. Biol.* **2003**, *521*, 1.

nitric oxide, prostaglandins, excitatory amino acids, and proinflammatory cytokines.[10, 11] Indeed, glial activation and subsequent release of proinflammatory mediators are now implicated in initiating and maintaining enhanced pain states.[10] Several of these cytokine mediators and the receptor-mediated events that they initiate, have been well characterized.

Among the most important of these are the toll-like receptors (TLRs), more importantly, TLR4.[12, 13] The TLRs are a family of transmembrane proteins that recognize a diverse range of chemical space occupied by exogenous and endogenous substances that are considered to function as danger signals, and induce activation of the innate immune system aimed at defending the survival of the host. TLR4 is especially intriguing with respect to glial activation. It has been extensively characterized in that it is the toll-like receptor that recognizes LPS (the lipopolysaccharide of gram-negative bacteria such as *E. coli* and Salmonella) and other endogenous danger signals released by damaged, dying, and dead neurons and other cells. It is clear that endogenous danger signals created as a result of nerve injury can cause chronic activation of glia via TLR4, and thereby establish a drive to maintain neuropathic pain.[4] Thus, targeting glia, specifically at their TLRs, may have potential clinical utility.

Given these considerations, the development of a glial cell activation/TLR4 antagonist could be quite useful. Professor Linda Watkins and her group at the Department of Psychology and the Center for Neuroscience at the University of Colorado at Boulder sought a blood brain barrier permeable, small molecule class of compounds that could block TLR4. Their

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[11] Watkins, L. R., Milligan, E. D., Maier, S. F. *Trends Neurosci.* **2001**, *24*, 450-455.

[12] Tanga, F., Nutile-McMenemy, M., DeLeo, J. *Proc. Natl. Acad. Scie. U. S. A.* **2005**, *102*, 5856 – 5861.

[13] Kim, D., Kim, M., Cho, I., Kim, M., Lee, S., Jo, E., Choi, S., Park, K., Kim, J., Akira, S., Na, H., Oh, S., Lee, S. *J. Biol. Chem. 2007, J. Biol. Chem.* **2007**, *282*, 14975 – 14983.

attention was drawn to the work of Hong et al.[14, 15, 16] and Lu[17] et al. which demonstrated the blockade of LPS activity by the opioid receptor antagonist naloxone. More importantly, it was documented that naloxone blocked microglial activation by LPS. Interestingly, the Hong group found that the actions of naloxone on LPS-induced microglial activation were not mediated by classical neuronal opioid receptors because both the opioid active (-)-isomer and the opioid inactive (+)-isomer of naloxone exerted identical inhibitory effects.[15] The observation that the (+)-isomer inhibited LPS-induced microglial activation was significant for it strongly suggested that naloxone must be acting through some receptor other than the classical opioid receptor, which only binds the (-)-isomer.

The Watkins group hypothesized that naloxone could bind to TLRs in addition to their well-known binding to classical opioid receptors. To test this, they studied the effects of (+)-naloxone on LPS-induced activation of TLR4; and to their delight, (+)-naloxone was successful in antagonizing TLR4 activation.[4] These data not only demonstrate novel receptor antagonistic action of (+)-naloxone, but reinforce the critical role of TLR4 receptors on glia in neuropathic pain.

The ground breaking discovery that opioid agonists activate TLR4 and an opioid antagonist would nonstereoselectively block TLR4 activation, suggests that the TLRs may lie at the intersection between neuropathic pain and opioid-induced glial activation. Thus the exciting potential exists that unnatural enantiomers of opioids may be uniquely positioned to suppress opioid induced glial activation, and yet not compromise the pain-suppressive effects of opioid

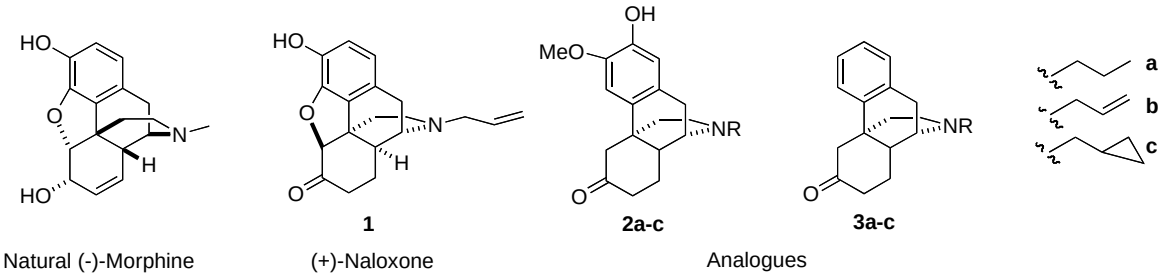
[14] Chang, R., Hong, J. S. *J. Brain Res*. **2000**, *854*, 224.

[15] Liu, B., Du, L. Hong, J. S. *J. Pharmacol. Exp Ther*. **2000**, *293*, 607.

[16] Liu, B., Du, L., Hong, J. *J. Pharmacol. Exp. Ther*. **2000**, *295*, 125.

[17] Lu, X., Bing, G., Hagg, T. *Neuroscience* **2000**, *97*, 285.

agonists on neurons. Therefore, the aim of our research is to synthesize a novel class of compounds, **2a-c**, **3a-c**, similar in structure to (+)-naloxone (**1**) and study their ability to suppress opioid-induced glial activation (Scheme 01).



Scheme 01. Structures of (-)-Morphine, (+)-Naloxone, and desired analogues.

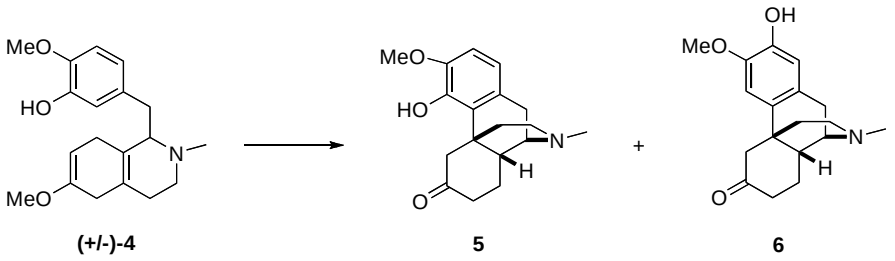
1.1 SYNTHETIC STRATEGIES FOR THE PREPARATION OF NOVEL MORPHINAN-LIKE COMPOUNDS

Our strategy for the synthesis of our desired analogs takes advantage of the Grewe cyclization of 1-benzyl-hexahydroisoquinolines, which is a well-established route to morphinans and a key reaction in the total synthesis of opium alkaloids by Kenner Rice. In the original work of Grewe and Friedrichsen[18] and Morrison et al.[19] it was found that cyclization of (±)-**4** provides a mixture of **5** and **6** with the desired compound **5** required for the synthesis of the natural opium alkaloids as the minor product (Scheme 02). Rice and coworkers developed a blocking strategy for the synthesis of the desired compound[20]; however we wished to exploit the inherent preference for the formation of **6** to prepare novel morphinan-like structures as described below.

[18] Grewe R., Friedrichsen W. *Chem Ber.* **1967**, *100*, 1150.

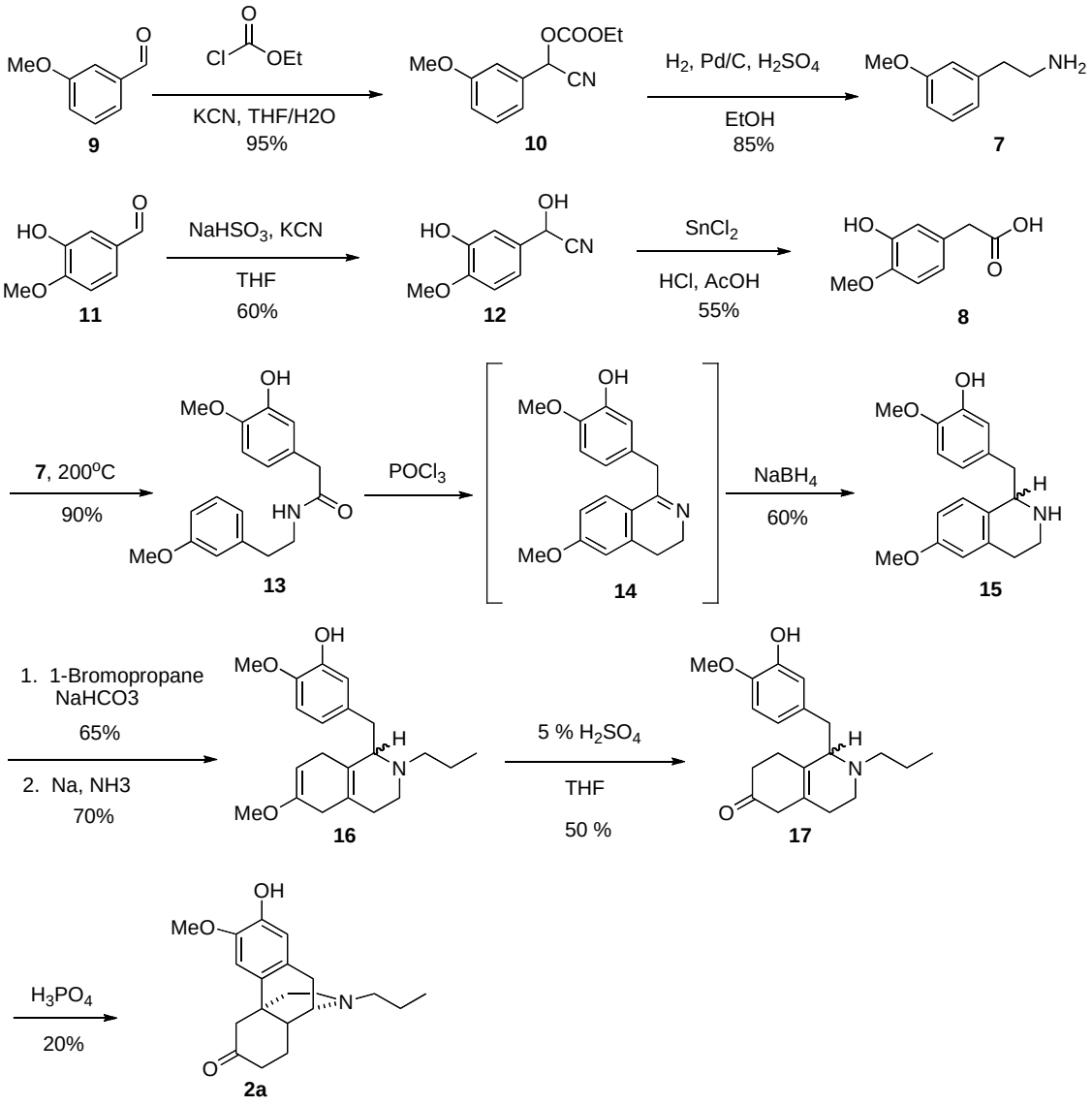
[19] Morrison G.C., Waite R.O., Shavel J. *Tetrahedron Lett.* **1967**, 4055.

[20] Rice K. C. *J. Org. Chem.* **1980**, *45*, 3135 – 3137.



Scheme 02. Grewe cyclization of (±)-**4** provided the undesired compound **6** as the major isomer.

Our efforts began with the preparation of amine **7** and acid **8** as illustrated in Scheme 03. Compound **7** was prepared from commercially available *m*-anisaldehyde **9** via hydrogenation of the cyanoformate intermediate **10** at 50 psi. Treating behzaldehyde **11** with sodium bisulfite then potassium cyanide produced cyanohydrin **12** which was reduced to **8** with tin (II) chloride in acid. Direct condensation of amine **7** and acid **8** at 200 ^{o}C readily afforded amide **13** in 90% yield. Bischler-Napieralski cyclization of **13** and in situ reduction of the intermediate imine (**14**) with sodium borohydride generated (±)-**15**. Alkylation of the secondary amine with sodium bicarbonate and 1-bromopropane (60 %) followed by birch reduction with sodium metal in ammonia provided (±)-**16** (70 %) yield. Formation of ketone (±)-**17** from the enol ether was accomplished with dilute acid in THF (50 %), and Grewe cyclization of (±)-**17** generated (±)-**2a** in a yield of only 20%.



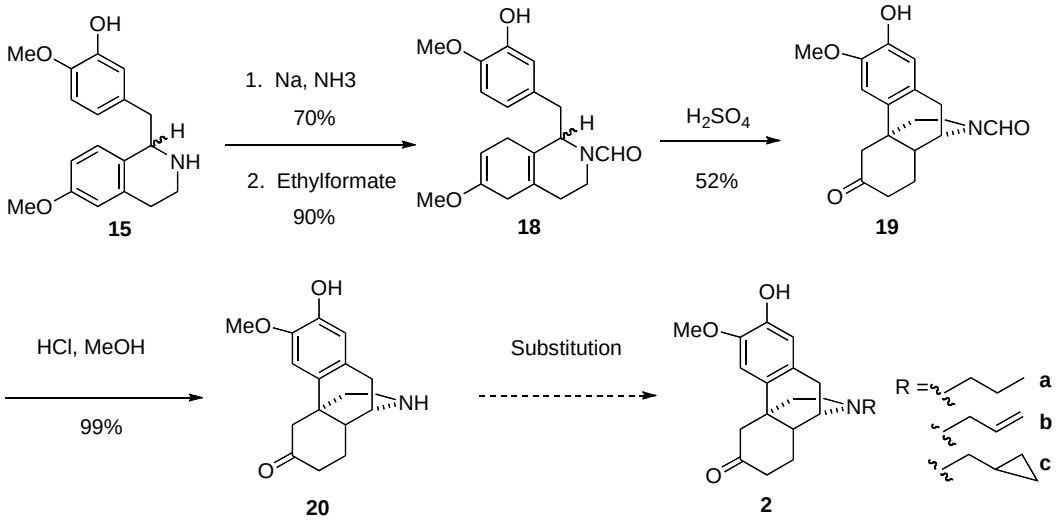
Scheme 03. Synthetic route to compound (±)-**2a**.

Due to the low yield of the cyclization of **17**, we studied the optimization of our key reaction. The Grewe cyclization of appropriately substituted hexahydroisoquinolines is greatly facilitated by the presence of an electron withdrawing group on the amine nitrogen and a hydroxy group at the para-position to the point of ring closure.[21] Since the work of Leimgruber[22]

[21] Brossi A., Schmidhammer H. *Can. J. Chem.* **1982**, *60*, 3055 – 3060.

[22] Leimgrber W., Moasci E. **1972**, US Pat 3634429.

and later Kenner Rice[20] had shown that N-acyl amines undergo Grewe or Grewe-like cyclization in a much more facile manner than the corresponding amines, we opted to study the readily removable N-formyl substituent in the Grewe cyclization. Therefore, Birch reduction of compound (±)-**15** was followed by formylation with neat ethyl formate to provide (±)-**18** (Scheme 04).



Scheme 04. Synthesis of (±)-**19**.

Indeed, Grewe cyclization of (±)-**18** with concentrated sulfuric acid in ether leads to (±)-**19** in an improved yield of 52 %. Isolation of (±)-**20**·HCl was accomplished nearly quantitatively by treating (±)-**19** with a refluxing solution of 4:1 MeOH/con. HCl. Substitution of the amine hydrogen with propyl, allyl or cyclopropyl methyl is next, and is not expected to give rise to any difficulties.

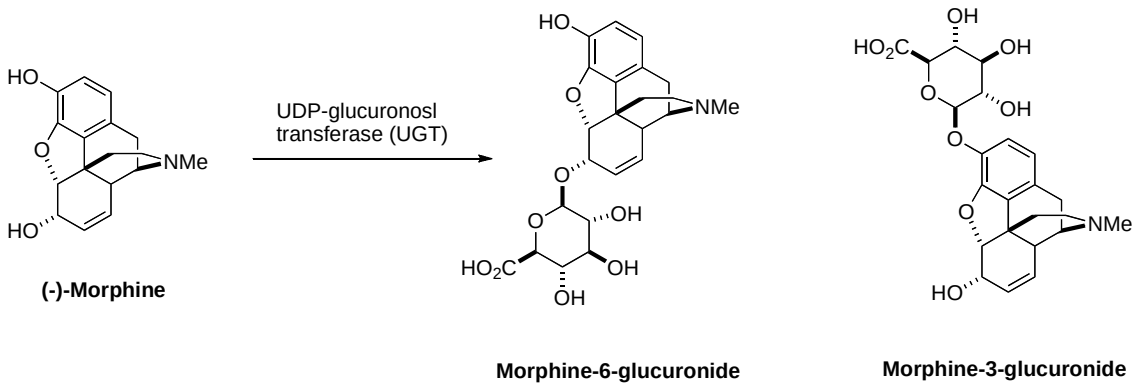
1.2 SYNTHETIC PREPARATION OF THE AROMATIC UNSUBSTITUTED SERIES

Morphine is principally metabolized in the liver by glucronidation. In humans, about 44 – 55 % of morphine is metabolized to morphine-3-glucuronice (M3G), 9 – 15 % to morphine-6-glucoronide (M6G), 8 – 10 % excreted as morphine and the remainder converted into numerous minor metabolites[23, 24] (Scheme 05). Similarly, (+)-Naloxone (Scheme 01) is glucuronidated in the liver at its phenolic site. (+)-Naloxone would be an ideal drug candidate for our purposes, however, metabolism of this compound happens too quickly in humans; its half-life is only about 60 minutes.[25] Because of this, we are interested in developing compounds lacking substitution on the aromatic ring.

[23] Anderson G., Christrup L. *J. Pain Sympton Manage*, **2003**, *25*, 74 – 91.

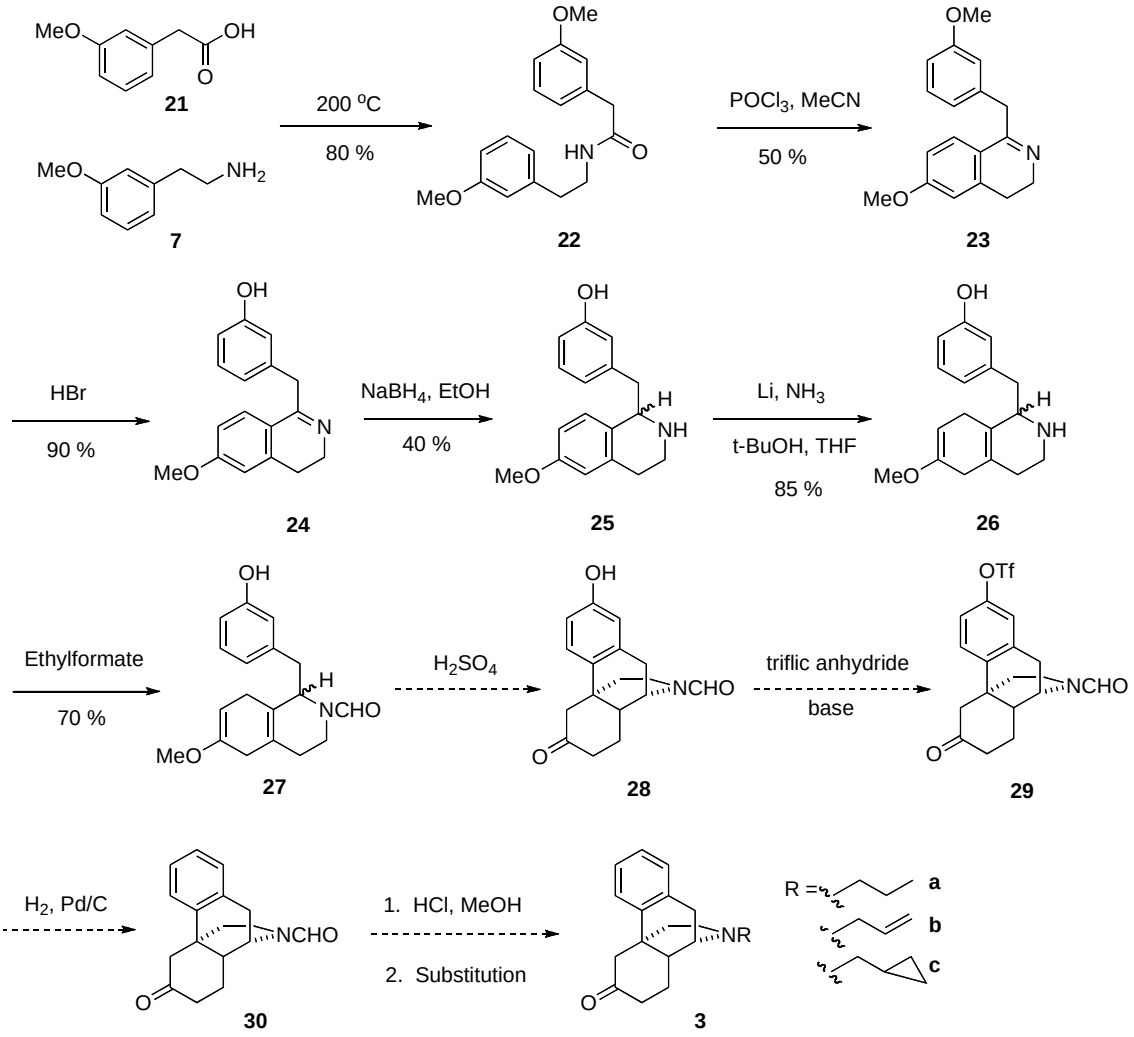
[24] Christrup L. *Acta Anaesthesiol Scand*, **1997**, *41*, 116-122.

[25] Ngai, S. H., Berkowitz, B., Yang, J., Hampstead, J., Spector, S. *Anesthesiology*, **1976**, *44*, 398-401.



Scheme 05. Glucronidation of morphine occurs in the liver via the enzyme UDP-glucoronosyl transferase (UGT).

The synthesis of the aromatic unsubstituted morphinan series of compounds proceeded as follows. Condensation of commercially available *m*-hydroxyphenylacetic acid **21** with amine **7** afforded amide **22** (80 %, Scheme 06). Beischler-Napieralski cyclization of amide **22** generated the 3,4-dihydroisoquinoline **23** (50 %), which underwent smooth O-demethylation with 48 % HBr to afford the phenolic 3,4-dihydroisoquinoline **24** in good yield (90 %). Reduction of imine **24** was carried out with $NaBH_4$, to provide tetrahydroisoquinoline (±)-**25** (40 %). Birch reduction of (±)-**25** afforded diene (±)-**26** (85 %), which was N-formylated with neat ethyl formate to (±)-**27** (70 %). The Grewe cyclization of (±)-**27** is expected to go smoothly in concentrated sulfuric acid in ether at room temperature to give (±)-**28**, which could then be triflated and reduced to intermediate (±)-**30**. Acid hydrolysis of (±)-**29** followed by substitution is anticipated to complete the synthesis of compounds (±)-**3a-c**.

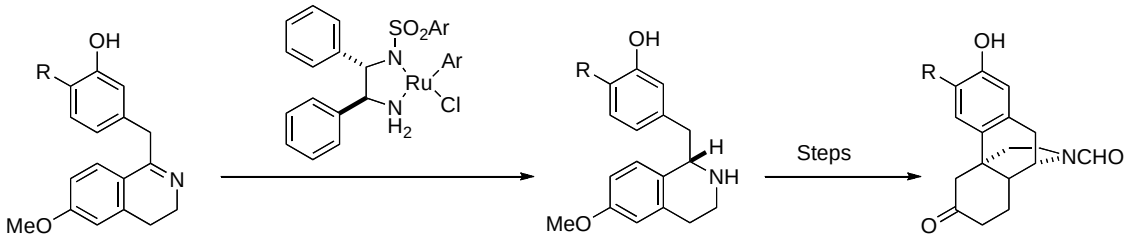


Scheme 06. Synthesis of compounds (±)-**3a-c**.

1.3 Future Directions: Asymmetric Transfer Hydrogenation, A Route to the (+)-isomers

Ultimately, once this synthesis is complete, it is our desire to investigate a method of producing these compounds as the unnatural enantiomers. These enantiomers would not share the specific actions of the natural (-)-isomers, and would be opioid receptor innactive. We have

focused our attention on the work of Kenner Rice[26] who has prepared asymmetric dihydroisoquinolines in good yield and high ee utilizing a Noyori chiral Ru catalyst.[27] Based on this work, we are encouraged to employ the enantioselective reduction of the cyclic imines **14** and **24** with a chiral Ru (II) complex of suitable design as illustrated in Scheme 07.



14: R = OCH_3
24: R = H

(S)-**15:** R = OCH_3
(S)-**25:** R = H

(+)-**19:** R = OCH_3
(+)-**28:** R = H

Scheme 07. Asymmetric transfer hydrogenation of cyclic imines **14** and **24** catalyzed by a chiral Ru (II) complex.

[26] Personal reference.

[27] Uematsu, N., Fujii, A., Hashiguchi, S., Ikariya, T., Noyori, R. *J. Am. Chem. Soc.* **1996**, *118*, 4916 – 4917.

CHAPTER TWO:

THE SYNTHESIS OF CORTICOSTERONE-GLUCURONIDE

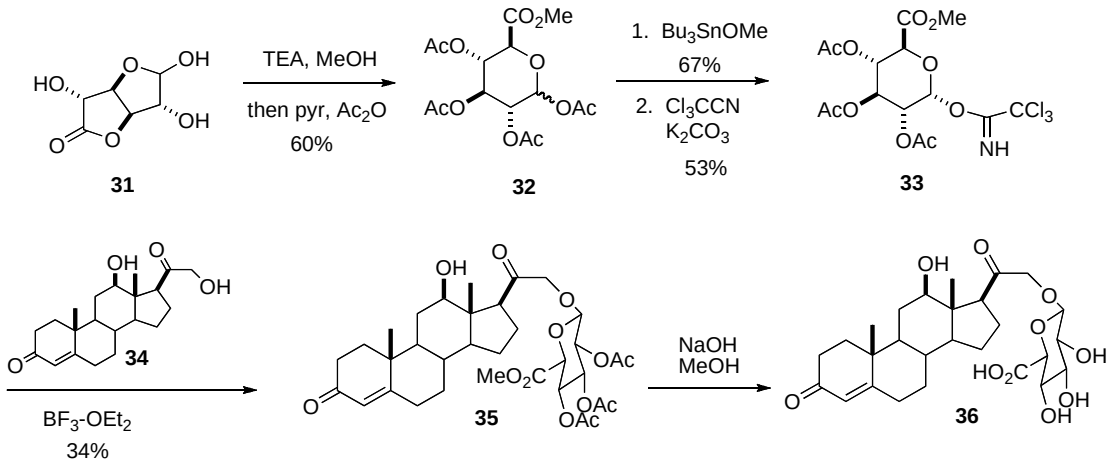
Understanding the role of the metabolism of neurotransmitters and neuromodulator metabolites is required in order to develop a deeper understanding of brain functions. Neurosteroids are an important class of neuromodulators that can either activate or inactivate neuron-neuron communications, thereby mediating many brain functions.[28] For example, it has been known for some time that the neurosteroid corticosterone, the rat version of human adrenal cortisol, displays an intense immunospuppresive effect at the time of delivery. However, recent work has shown that it produces dramatic glial and immune activation and a proinflammatory response later in time. Corticosterone, like morphine, is glucuronidated in its metabolic pathway. The aim of this research was to synthesize Corticosterone-glucuronide (CortG) and test whether the glucuronidation is the cause of the eventual proinflammatory effects of corticosterone.

2.1 SYNTHETIC ROUTE TO CORTG

Scheme 08 illustrates the synthesis of CortG and commences with the treatment of commercially available D-(+)-glucuronolactone **31** with triethylamine in methanol followed by treatment with pyridine and acetic anhydride to generate **32** in 60 % yield. Deacylation of **32** at the anomeric position was accomplished with tributyltin methoxide (67 %) followed by subjection to potassium carbonate and trichloroacetonitrile to provide the corresponding trichloroacetimidate **33** (53 %). Compound **33** was coupled to commercially available corticosterone **34** with boron trifluoride ether complex to provide **35** (34 %). Removal of the

[28] Stoffel-Wagner, B., *Ann. NY Acad. Sci.*, **2003**, *1007*, 64-78.

acyl groups of **35** was accomplished with sodium hydroxide in methanol to provide the desired carboxylic acid **36** in a 10 % yield.



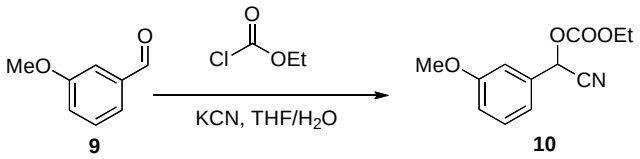
Scheme 08. Synthesis of CortG **36**.

CHAPTER 3:

EXPERIMENTAL METHODS AND CHARACTERIZATION

3.1 PROCEDURES AND CHARACTERIZATION OF COMPOUNDS

O-(Ethoxycarbonyl)-3-methoxymandelonitrile (10)[29]:

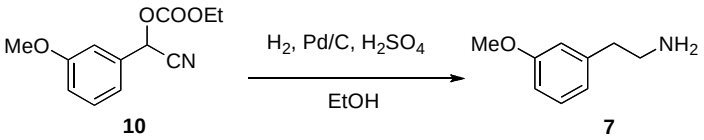


9 **10**

To a stirred solution of 30 g (220 mmol) of m-anisaldehyde **9** and 26.3 g (242 mmol, 23.3 ml) of ethyl chloroformate in 44 ml of THF cooled on an ice bath, was added 15.8 g (242 mmol) of KCN dissolved in 30 ml of H_2O in one portion. The reaction mixture was stirred for 4 h at 5 ˚C and then slowly warmed to room temperature overnight. 60 ml of water was added, the mixture was transferred to separatory funnel, and the aqueous solution was extracted with ether (4 x 25 ml). The organic extracts were washed with fresh water, dried over anhydrous Mg_2SO_4 and evaporated to 49.3 g (95 % crude yield) of a yellow oil. The crude material was carried on without purification. ^{1}H NMR (500 MHz, $CDCl_3$): NMR δ 7.34 (1 H, t, J = 8 Hz), 7.10 (1 H, broad d, J = 7.7 Hz), 7.03 (1 H, t, J = 2 Hz), 6.99 (1 H, dd, J = 2.5, 8.5), 6.21 (1 H, s), 4.27 (2 H, m), 3.82 (3H, s), 1.32 (3 H, t, J = 7 Hz).

[29] Rapaport H, Kashdan D, Schwarz J. *J. Org. Chem.* **1982**, *47*, 2638 – 2643.

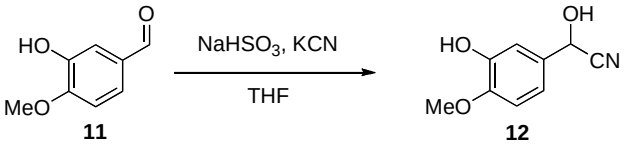
2-(m-Methoxyphenyl)ethylamine (7) [29]**:**



10 **7**

500 ml of absolute ethanol and 2.5 g of 5 % Pd/C catalyst was added to a 2000 ml Parr Shaker reaction vessel. 20 g (85 mmol) of cyanoformate **10** was transferred to the reaction vessel with the aid of 350 ml of absolute ethanol, followed by 16.8 g (170 mmol, 9.1 ml) of concentrated H_2SO_4. The mixture was shaken under 50 psi for 12 h. Celite was added to the mixture and this solution was filtered through a thin celite pad. The solvent was evaporated to a yellow oil, water (50 ml) was added, and the cooled aqueous solution was made alkaline with 4 M sodium hydroxide. The solution was extracted with 4 x 30 ml of ether, and organic extracts were combined, dried over anhydrous Mg_2SO_4, and evaporated to a yellow oil which was Kugelrohr distilled to afford 11.2 g (85 %) of phenethylamine **7** as a thin, clear oil. ^{1}H NMR (500 MHz, $CDCl_3$): NMR δ 7.20 (1 H, t of d, J = 1, 7.5 Hz), 6.78 – 6.73 (3 H, m), 3.78 (3 H, s), 2.94 (2 H, t, J = 7 Hz), 2.70 (2 H, t, J = 7 Hz), 1.05 (2 H, broad s).

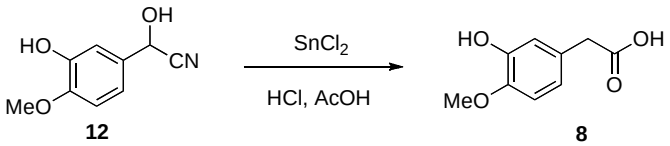
Cyanohydrin (12)[30]**:**



20 g (131 mol) of 3-hydroxy-4-methoxy benzaldehyde was added in small portions at 50 ˚C to 40 ml of 40 % aqueous $NaHSO_3$ and 60 ml water. This mixture was cooled rapidly to -5 ˚C, treated dropwise at -5 ˚C with 17.1 g (263 mmol) KCN dissolved in 26 ml of water during 1 – 1.5 h. The solution was stirred for 0.5 h, acidified with 5 N H_2SO_4 at -5 ˚C (pH = 2). The mixture becomes white and thick upon addition to 5 M H_2SO_4. The mixture was extracted after another 0.5 h of stirring with ether (4 x 30 ml). The organic extracts were combined, washed with fresh water, dried over Mg_2SO_4 and evaporated to a yellow solid. The crude residue was recrystallized from dichloroethane and dried to yield 19.3 g (82 %) of the cyanohydrin as a light yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6): NMR δ 9.25 (1 H, s), 6.95 – 6.83 (3 H, m), 5.57 (1 H, d J = 6 Hz), 3.76 (3 H, s).

[30] Grewe R., Fischer H. *Chem. Ber*. **1963**, *96*, 1520 – 1528.

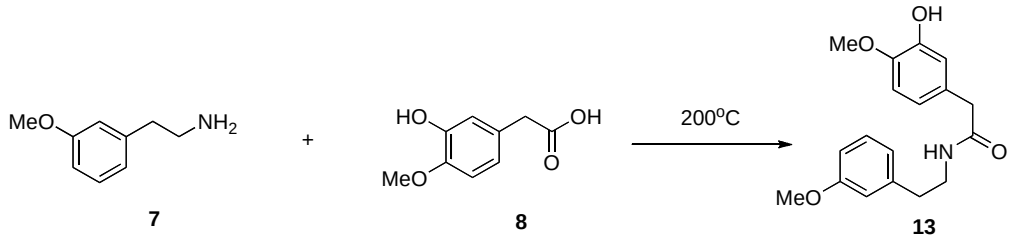
(3-Hydroxy-4-methoxyphenyl)acetic Acid (8)[30,31]**:**



A stirring solution of 10 g (56 mmol) of **12** in 12.8 ml of AcOH and 15.9 g (84 mmol) $SnCl_2 \cdot 2\ H_20$ was treated with 16.3 ml of concentrated HCl. The mixture was heated for 4 h at 110 ˚C, cooled to room temperature, filtered through celite to get rid of excess $SnCl_2$, and diluted with 20 ml of water. This aqueous solution was extracted continuously with $CHCl_3$ for 12 h. The organic extracts were evaporated to a light orange, crude solid which was heated to solution in 5 ml of 2-propanol and treated with 1 ml of concentrated aqueous NH_3 to afford crystalline material almost immediately. The slurry was cooled to room temperature, and the solid was filtered, washed with of 2-propanol, and dried to afford 11.5 g of light pink $\mathbf{8} \cdot NH_3 \cdot H_2O$ which was heated to solution in about 20 ml of H_2O and treated with 20 ml of concentrated HCl. After the mixture was cooled on an ice bath, the acid was filtered, washed with cold 6 N HCl, and dried to afford 4.7 g (46 %) of **8** as a white solid, mp 129 – 130.5 ˚C; ^{1}H NMR (500 MHz, $CDCl_3$): NMR δ 10.3 (1 H, broad s), 6.84 (1 H, d, J = 2 Hz), 6.79 – 6.73 (2 H, m), 5.59 (1 H, broad s), 3.86 (3 H, s), 3.54 (2 H, s).

[31] Rice K, Brossi A. *J. Org. Chem.* **1980**, *45*, 92 – 601.

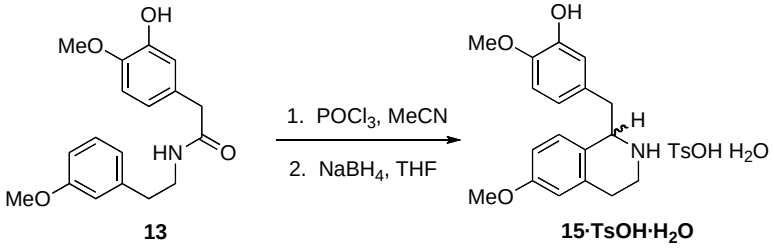
Amide (13)[30]**:**



7 **8** **13**

A mixture of 5.5 g (30 mmol) of dry acid **8** and 4.6 g (30 mmol) of dry amine **7** in a 100 ml flask was vac/nitrogen purged (4 x). The flask was placed in a silicon oil bath and stirred and heated for 2 h (bath temperature 195 – 200 ˚C) while passing a nitrogen sweep over the melt. The clear melt was cooled to room temperature, heated to solution in about 4 ml of AcOH, and diluted with 4 ml of H_2O. The solution was slowly cooled to room temperature, placed on an ice bath, and when crystallization was complete, the orange solid was filtered, and washed with 1:1 AcOH/H_2O. The solid was recrystallized from ethyl acetate to produce 6.2 g (65 %) of amide **13** as a white solid, mp 112 – 115 ˚C; ^{1}H NMR (500 MHz, $CDCl_3$): NMR δ 7.14 (1 H, m), 6.76 – 6.71 (3 H, m), 6.64 – 6.57 (3 H, m), 5.63 (1 H, s), 5.39 (1 H, broad s), 3.87 (3 H, s), 3.76 (3 H, s), 3.46-3.39 (4 H, m), 2.68 (2 H, t, J = 7 Hz).

Isoquinoline (±)-15·TsOH·H_2O[30,32]:



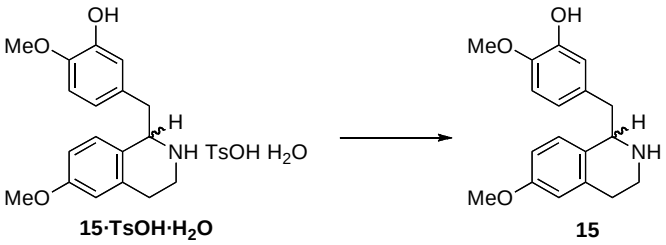
13 **15·TsOH·H_2O**

A stirred, refluxing solution of 2.0 g (6.3 mmol) of amide **13** in 32 ml of acetonitrile was treated with 3.8 ml (40.6 mmol) of $POCl_3$ during 10 min (exothermic reaction). The mixture was refluxed for 1 hr and cooled to room temperature. The solvent and $POCl_3$ was distilled off to leave behind a red foam which was heated to solution in 9 ml of water and allowed to reflux for 1 h (exothermic reaction, add water slowly). The mixture was cooled until a yellow oil separated, 6.4 ml of THF was added, and passage of nitrogen *through* the solution was begun and continued for the duration of the reaction. The solution was cooled to 0 – 5 ˚C during 0.5 h, and sufficient concentrated aqueous NH_3 was added (T < 15 ˚C) to give a final pH of 8.5 – 9.5. The reaction mixture, which contained a yellow solid, was cooled to 0 ˚C, and 48 mg (12.7 mmol) of $NaBH_4$ was quickly added in portions. When the addition of $NaBH_4$ was complete, the mixture was stirred at 0 ˚C for 0.5 h, and then at room temperature for 2 h. The yellow reaction mixture turns tan upon addition of $NaBH_4$. The flow of nitrogen was discontinued, 10 ml of $CHCl_3$ was added along with 20 ml of water. Stirring was continued until the solids had dissolved. The biphasic mixture was transferred to a seperatory funnel, the organic layer was collected, and the aqueous reextracted with $CHCl_3$ (4 x 10 ml). The combined, wet $CHCl_3$ extracts were evaporated to a grey-purple foam and heated in 11 ml of water containing 1.1 g

[32] Rice, K. *J. Org. Chem.* **1980**, *45*, 3135 – 3137.

(5.8 mmol) of $TsOH \cdot H_2O$ until the grey-purple foam turned into a yellow solid. After being cooled to 5 ˚C for 1 h, the yellow solid was filtered and washed with cold water to obtain 2.0 g (65 %) of (±)-**15**·$TsOH \cdot H_2O$, which was carried on without purification. ^{1}H NMR (500 MHz, $CDCl_3$): NMR δ 7.50 (2 H, d, J = 8 Hz), 7.06 (2 H, d, J = 8 Hz), 6.92 (1 H, d, J = 2 Hz), 6.83 – 6.53 (5 H, m), 4.63 (1 H, m), 3.79 (3 H, s), 3.76 (3 H, s), 3.50 – 3.30 (2 H, m), 3.15 (2 H, d, J = 7 Hz), 3.0 (2 H, m).

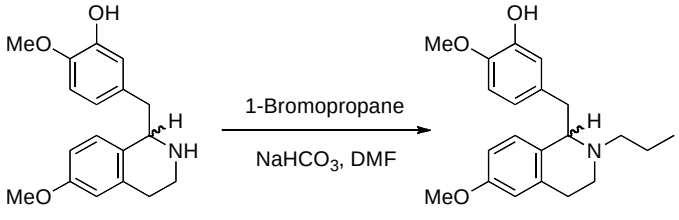
Isoquinoline (±)-15[31,32]**:**



A mixture of 2.0 g (4.1 mmol) of (±)-**15**·$TsOH \cdot H_2O$ and 2 ml of methanol was heated to solution. To the stirred mixture was added *in order* 10 ml of $CHCl_3$, 1.33 ml of concentrated aqueous NH_3, and 6.7 ml of water. The water layer was decanted into a seperatory funnel while the thick, cloudy organic layer was transferred to a beaker. The aqueous layer was extracted with $CHCl_3$ (4 x 5 ml) and combined with the cloudy organic layer from above. This $CHCl_3$ mixture was filtered through a fritted funnel to collect a white solid, and the mother liquor was evaporated to a brown residue. This brown residue and the white solid collected was heated in about 10 ml of butyronitrile until the mixture was tan and homogeneous. This thick solution was cooled to room temperature, then to 0 ˚C for 1 h. The tiny crystals were filtered and washed with ice-cold butyronitrile to provide 2.5 g (60 %) of (±)-**15** as a white solid, mp 199 – 201 °C; ^{1}H

NMR (500 MHz, $CDCl_3$): NMR δ 7.14 (1 H, d, J = 8.5 Hz), 6.84 (1 H, s), 6.79 (1 H, d, J = 8.14 Hz), 6.75 – 6.69 (2 H, m), 6.62 (1 H, d, J = 2.6), 5.63 (1 H, s, broad), 4.09 (1 H, dd, J = 3.5, 10.3 Hz), 3.86 (3 H, s), 3.77 (3 H, s), 3.20 – 3.12 (2 H, m), 2.90 – 2.70 (4 H, m).

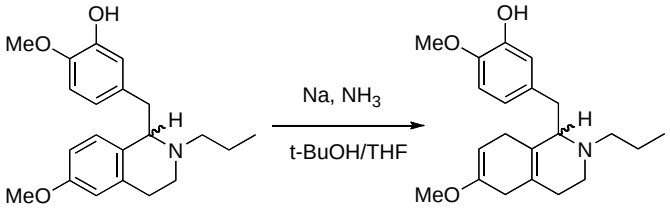
Amine (±)-37:



15 **37**

1.3 g (4.4 mmol) in 17 ml of DMF was treated with 1.8 g (21.8 mmol) of $NaHCO_3$ and 0.4 ml (4.8 mmol) of distilled 1-bromopropane. The solution was heated to 80 – 90 ˚C and allowed to stir for 18 h. The mixture was cooled to room temperature, diluted with 3 ml of H_2O and extracted with $CHCl_3$ then brine. The organic extracts were combined, dried over Mg_2SO_4, and evaporated to a brown residue that was purified by column chromatography in 97:2:1 $CHCl_3$/MeOH/con. NH_3 (Rf = 0.30, stained in CAM) to provide 1.1 g (73 %) of the desired alkylated product as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): NMR δ 6.76 (1 H, d, J = 2 Hz), 6.72 (1 H, d, J = 8 Hz), 6.60 – 6.53 (4 H, m), 5.63 (1 H, broad s), 3.85 (3 H, s), 3.79 (1 H, t, J = 7 Hz), 3.74 (3 H, s), 3.21 (1 H, m), 2.99 – 2.82 (4 H, m), 2.66 (1 H, q), 2.50 (3 H, t, J = 7.5 Hz), 1.44 (2 H, m), 0.79 (3 H, t, J = 7.5 Hz). ^{13}C (75 MHz, $CDCl_3$): NMR δ 157.88, 145.39, 145.08, 135.71, 133.69, 130.32, 129.49, 121.34, 116.03, 113.37, 111.81, 110.49, 62.72, 56.16, 55.92, 55.36, 43.75, 41.49, 25.47, 21.25, 11.99.

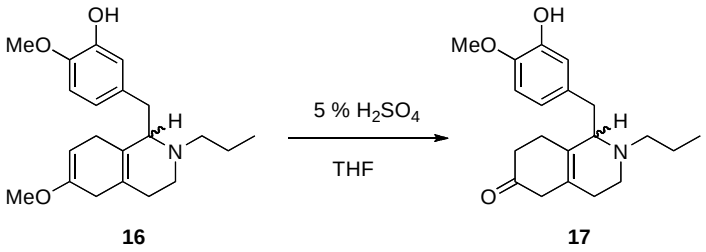
Diene (±)-16:



37 **16**

10.5 ml of ammonia was condensed into a 3 neck flask in a -78 ˚C (dry ice/acetone) bath under a nitrogen atmosphere, and 423 mg (18.4 mmol) of sodium metal was added. To this blue solution, was added slowly 523 mg (1.5 mmol) of (±)-**37** dissolved in 10.5 ml of a 1:1 mixture of t-BuOH/THF at -78 ˚C. The dry ice/acetone bath was switch to a $CHCl_3$/liquid nitrogen bath and the reaction mixture was stirred between -55 and -65 ˚C for 2 h. The cold bath was dropped and the mixture was allowed to warm to room temperature. The white residue left over was dissolved in 5 ml of water, transferred to a separatory funnel, and extracted with $CHCl_3$ (4 x 2 ml). The organic extracts were combined, dried over Mg_2SO_4, and evaporated to 31 mg (59 %) of (±)-**16** as a yellow residue which was carried on without purification. ^{1}H NMR (500 MHz, $CDCl_3$): NMR δ 6.82 (1 H, d, J = 2 Hz), 6.72 (2 H, d, J = 8.2), 6.88 (1 H, dd, J = 2, 8), 5.80 (1 H, broad s), 4.58 (1 H, t, J = 3.5 Hz), 3.83 (3 H, s), 3.52 (3 H, s), 3.08 (1 H, m), 2.99 (1 H, m), 2.72 – 2.39 (9 H, m), 2.18 (1 H, m), 1.63 (1 H, broad d, J = 16.8 Hz), 1.35 (2 H, m), 0.73 (3 H, t, J = 7.4 Hz). ^{13}C (75 MHz, $CDCl_3$): NMR δ 152.63, 145.36, 144.93, 124.37, 120.87, 115.59, 110.56, 90.66, 63.32, 56.21, 55.62, 54.04, 43.31, 37.54, 33.72, 30.14, 29.23, 25.89, 22.92, 21.24, 11.98.

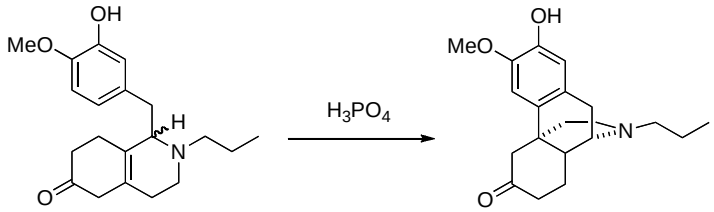
Ketone (±)-17:



16 **17**

322 mg of (±)-**16** (0.9 mmol) in 32 ml of THF was treated with 8.2 ml of a 5 % aqueous solution of H_2SO_4. The reaction mixture was allowed to stir for 72 h at room temperature, washed with saturated $NaHCO_3$, dried over Mg_2SO_4, and evaporated to a dark yellow oil. The crude was purified by column chromatography in 97:2:1 $CHCl_3$/MeOH/con. NH_3 (Rf = 0.2, stained in CAM) to afford 158 mg (51 %) of the pure ketone as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): NMR δ 6.82 (1 H, d, J = 2 Hz), 6.72 (1 H, d, J = 8.2 Hz), 6.66 (1 H, dd, J = 2.0, 8.2 Hz), 3.84 (3 H, s), 3.11 (1 H, broad t, J = 5.9 Hz), 3.01 (1 H, m), 2.83 – 2.67 (4 H, m), 2.63 (1 H, dd, J = 5.7, 13.9), 2.47 – 2.30 (4 H, m), 2.14 (3 H, m), 1.70 (1 H, broad d, J = 17.7 Hz), 1.39 (2 H, m), 0.78 (3 H, t, J = 7.4 Hz). ^{13}C (75 MHz, $CDCl_3$): NMR δ 210.81, 145.52, 145.15, 131.37, 125.7, 120.81, 115.53, 110.61, 63.84, 56.19, 55.87, 44.28, 43.13, 39.18, 36.92, 29.85, 28.89, 26.64, 21.19, 12.02.

Amine (±)-2a[33]**:**

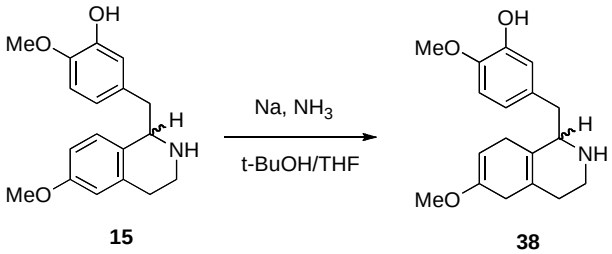


17 **2a**

100 mg (0.3 mmol) of amine (±)-**17** was placed into a cold finger and treated with 2 ml of 85 % H_3PO_4. The mixture was heated on a sand bath to about 80 ˚C and allowed to stir for 20 h while nitrogen was bubbled *through* the solution. The mixture was cooled to room temperature, diluted with 6 ml of cold water, and treated with concentrated NH_3 until pH = 8. The aqueous mixture was extracted with $CHCl_3$ (4 x 3 ml). The organic layers were combined, dried over Mg_2SO_4 and evaporated to provide the crude product as a brown residue which was purified by column chromatography in 95:4:1 $CHCl_3$/MeOH/con. NH_3 (Rf = 0.29, stained in CAM) to provide 21 mg of (±)-**2a** (21 %). ^{1}H NMR (500 MHz, $CDCl_3$): NMR δ 6.68 (1 H, s), 6.62 (1 H, s), 3.84 (3 H, s), 3.14 (1 H, broad s), 3.04 (1 H, dd, J = 2, 14 Hz), 2.91 (1 H, d, J = 18.6 Hz), 2.65 – 2.17 (10 H, m), 2.11 (1 H, td, J = 2.8, 12.3 Hz), 1.91 (2 H, m) 1.55 (2 H, m), 1.42 (1 H, dt, J = 2.4, 12.7 Hz), 0.92 (3 H, t, J = 7.4 Hz). ^{13}C (75 MHz, $CDCl_3$): NMR δ 209.89, 145.61, 144.32, 123.99, 113.65, 108.54, 57.09, 56.34, 55.34, 51.94, 44.69, 43.83, 41.81, 41.43, 41.27, 27.05, 24.30, 20.95, 12.21.

[33] Grewe R., Friedrichsen W. *Chem. Ber.* **1967**, *100*, 1550 – 1558.

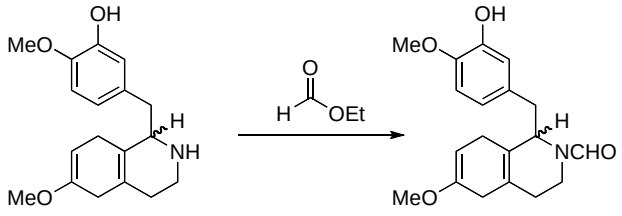
Amine (±)-38[32]:



15 **38**

27 ml of ammonia gas was condensed into a three-neck flask in a -78 ˚C bath (dry ice/acetone) and treated with 0.6 g (27.6 mmol) of sodium metal under nitrogen. 1.2 g (3.9 mmol) of (±)-**15** dissolved in 27 ml of a 1:1 mixture of t-BuOH/THF was added slowly to the blue solution. The dry ice/acetone bath was changed to a $CHCl_3$/liq nitrogen bath and the mixture was stirred between -55 and -65 ˚C for 2 h. The cold bath was dropped, and the reaction was allowed to warm to room temperature open to air. The white residue left over was diluted with about 8 ml of water and transferred to a separatory funnel. The aqueous mixture was extracted with $CHCl_3$ (4 x 5 ml), and organic extracts were combined, dried over Mg_2SO_4, and evaporated to 0.9 g (72 %) of (±)-**38** as a yellow solid. The crude was one spot by TLC in 90:9:1 $CHCl_3$/MeOH/con. NH_3 (Rf = 0.4, stained in CAM), and was carried on without purification. ^{1}H NMR (500 MHz, $CDCl_3$): NMR δ 6.80 – 6.76 (2 H, m), 6.68 (1 H, dd, J = 2, 8.2 Hz), 5.68 (1 H broad s), 4.65 (1 H, broad t, J = 2.7 Hz), 3.86 (3 H, s), 3.55 (3 H, s), 3.30 (1 H, broad d, J = 11.4 Hz), 3.08 – 2.87 (3 H, m), 2.79 (1 H, m), 2.67 - 2.48 (4 H, m), 2.0 (2 H, m).

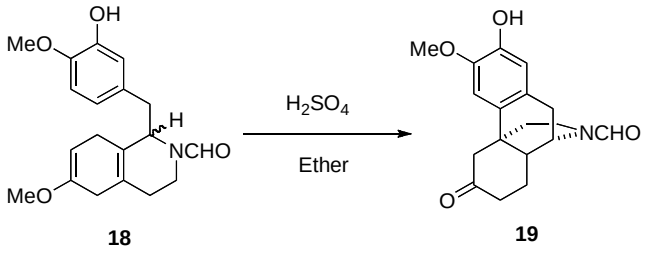
Formamide (±)-18[32]:



38 **18**

693 mg (2.3 mmol) of (±)-**38** was treated with 10 ml of freshly distilled ethylformate and refluxed for 12 h under nitrogen in a cold finger. The solvent was evaporated to a tan residue was flashed in 96:3:1 $CHCl_3$/MeOH/con. NH_3 (Rf = 0.3, stained in CAM) to yield 526 mg (69 %) of the desired (±)-**18** as a white foam. TLC of this material in showed two closely running spots corresponding to the two rotamers in a 2:1 ratio, which were apparent by the NMR spectrum. ^{1}H NMR (500 MHz, $CDCl_3$): NMR δ 7.93 (0.50 H, s), 7.41 (1 H, s), 6.74 – 6.66 (4 H, m), 6.51 (1 H, dd, J = 2, 8 Hz), 5.66 (1 H, s), 5.60 (0.5 H, s), 4.75 (0.5 H, t, J =6.5 Hz), 4.67 (1.5 H, dt, 3.5, 13.5), 4.40 (1 H, m), 3.83 (5 H, two s corresponding to methoxy group), 3.67 (1 H, dd, J = 9.4 Hz), 3.55 (5 H, two s corresponding to methoxy group), 3.39 (0.5 H, m), 3.10 – 2.90 (5 H, m), 2.80 – 2.57 (6 H, m) 2.18 (2 H, m), 1.90 (1 H, dd, J = 4, 17 Hz), 1.80 (0.5 H, dd, J = 4, 17 Hz).

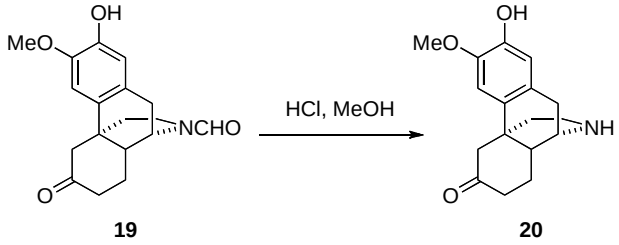
Formamide (±)-19:



18 **19**

To a well stirred suspension of 114 mg (0.35 mmol) of (±)-**18** and 3.46 ml of ether were added dropwise 2.21 ml (42 mmol) of 80 % H_2SO_4 at 0 ˚C under a nitrogen atomosphere during 1.5 h. The resulting solution was then allowed to stir at room temperature for 20 h. About 6 ml of ice-cold water was added to the yellow mixture and the aqueous solution was extracted with 1:1 $CHCl_3$/2-propanol (6 x 10 ml). The organic layers were combined, washed with fresh water, dried over Mg_2SO_4, and evaporated to give the crude product as a tan foam. The crude was purified by column chromatography in 90:1 MeOH/$CHCl_3$ (Rf = 0.25, stained in CAM) to isolate pure (±)-**19** as a white solid (52 %). TLC of this material appears as two very closely running spots corresponding to the two rotamers, in about a 1:1 ratio, apparent by the NMR spectrum. ^{1}H NMR (500 MHz, $CDCl_3$): NMR δ 8.17 (1 H, s), 8.01 (1 H, s), 6.72 (2 H, s), 6.62 (2 H, d, J = 6.2 Hz), 5.56 (2 H, d, J = 4.2 Hz), 4.81 (1 H, dd, J = 3.7, 5.8), 4.21 (1 H, dd, J = 4.9, 13.8 Hz), 3.85 (6 H, two s corresponding to methoxy groups), 3.31 (1 H, dd, J = 4.7, 13.5 Hz), 3.19 – 2.96 (5 H, m), 2.66 (2 H, m), 2.50 (1 H, td, J = 3.7, 13.3 Hz), 2.44 – 2.22 (4 H, m), 2.30 – 2.20 (2 H, m), 2.18 – 2.0 (2 H, m), 1.95 – 1.85 (1 H, m), 1.80 – 1.50 (9 H, m). ^{13}C (75 MHz, $CDCl_3$): NMR δ 203.56, 208.32, 160.74, 160.71, 146.13, 146.04, 144.95, 144.89, 127.99, 127.46, 127.38, 114.07, 113.94, 108.63, 56.36, 56.34, 52.94, 51.67, 51.63, 45.57, 44.04, 42.72, 42.51, 42.37, 42.18, 40.99, 40.93, 40.87, 40.15, 33.96, 32.51, 31.19, 26.53, 26.41.

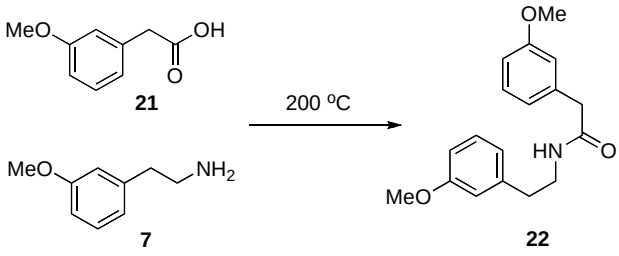
Amine (±)-20:



19 **20**

21 mg (0.07 mmol) of (±)-**19** was treated with about 1 ml of a 4:1 MeOH/HCl solution and refluxed under nitrogen for 16 h. The mixture was allowed to cool to room temperature, diluted with water, basified with con. NH_3, and extracted with 3:1 $CHCl_3$/2-propanol. The organic extracts were combined, dried over Mg_2SO_4, and evaporated to 17 mg of (±)-**20** as the free base. ^{1}H NMR (500 MHz, $CDCl_3$): NMR δ 6.68 (1 H, s), 6.63 (1 H, s), 3.84 (3 H, s), 3.28 (1 H, dd, J = 6, 3.2 Hz), 3.11 – 2.97 (2 H, m), 2.74 – 2.70 (2 H, m), 2.64 (1 H, td, J = 3, 12.7 Hz) 2.47 – 2.32 (3 H, m), 2.28 – 2.14 (3 H, m), 1.51 (2 H, qd, J = 4.9, 13 Hz), 1.43 (2 H, dt, J = 2.5, 12.5 Hz). ^{13}C (75 MHz, $CDCl_3$): NMR δ 209.56, 145.65, 144.43, 129.63, 128.78, 113.76, 108.67, 56.34, 52.30, 50.51, 44.51, 42.47, 41.85, 41.49, 38.06, 33.81.

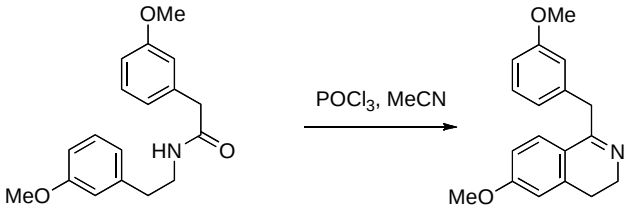
Amide (22):



A mixture of 10 g (60 mmol) of commercially available 3-methoxyphenylacetic acid **21** and 9.10 g (60 mmol) of pure amine **7** was heated to 200 ˚C in a silicon oil bath with a nitrogen

sweep. When the mixture was completely melted together, the melt was allowed to stir for 2 h, and cooled to room temperature. The melt was dissolved in about 15 ml of $CHCl_3$ and washed with fresh water and brine. The organic portion was dried over Mg_2SO_4 and evaporated to a yellow oil which was purified with column chromatography in 1:1 Hex/EtOAc (Rf = 0.33, stained in CAM) to isolate 13.9 g (78 %) of **22** as a clear oil. ^{1}H NMR (500 MHz, $CDCl_3$): NMR δ 7.20 (1 H, t, J = 8 Hz), 7.12 (1 H, t, J = 8 Hz), 6.8 (1 H, dd, J = 2.5, 8 Hz), 6.73 – 6.69 (3 H, m), 6.60 – 6.58 (2 H, m), 5.41 (1 H, broad s), 3.76 (3 H, s), 3.75 (3 H, s), 3.49 (2 H, s), 3.44 (2 H, q, J = 6.8), 2.68 (2 H, t, J = 6.9 Hz).

Imine (23·HBr)[33]**:**



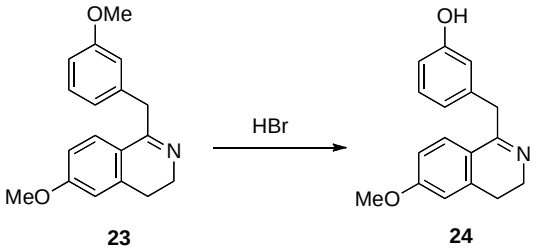
22 **23**

To a solution of 8 g (27 mmol) of **22** in 33 ml of acetonitrile were added 4.2 ml (45 mmol) of $POCl_3$. At room temperature nitrogen was passed through this mixture during 0.5 h. Then the mixture was refluxed for 1.0 h under nitrogen, evaporated by distillation, cooled to 0 – 5 ˚C and basified with con. NaOH. The aqueous mixture was extracted with CH_2Cl_2, and the organic extracts were combined, washed with fresh water and brine, dried over Mg_2SO_4, and evaporated to an oily residue which was dissolved in 5 ml of MeOH. To this solution, sufficient con. HBr was added to give pH of 1. Addition of ether and cooling to 0 ˚C overnight produced

[33] Brossi, A., Schmidhammer, H. *Can. J. Chem.* **1982**, *60*, 3055 – 3060.

7.35 g (76 %) of crude **23**·HBr, which was isolated as a light yellow solid by vacuum filtration and carried on without purification. ^{1}H NMR (500 MHz, $CDCl_3$): NMR δ 7.84 (1 H, d, J = 9 Hz), 7.19 (1 H, t, J = 8 Hz), 7.05 (1 H, t, J = 2 Hz), 6.93 (1 H, d, J = 7.7), 6.88 (1 H, dd, J = 2.5, 8.8), 6.81 – 6.73 (2 H, m), 4.59 (2 H, s), 3.96 (2 H, td, J = 3.4, 8 Hz), 3.88 (3 H, s) 3.79 (3 H, s), 3.04 (2 H, t, J = 7.8 Hz).

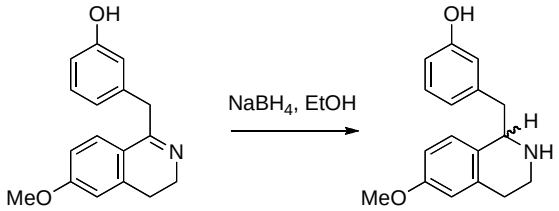
Imine (24·HBr)[33]**:**



23 **24**

A solution of 2.35 g (6.5 mmol) of **23**·HBr in 5.6 ml of con. HBr was refluxed for 50 min. and evaporated to a thick, reddish-brown oil which was dissolved in 1.5 ml of MeOH. This mixture was cooled on an ice bath, then Et_2O was added, and the resulting mixture was kept at 0 ˚C overnight to yield **24**·HBr as a tan, sticky solid which was isolated by gravity filtration. This material was dried thoroughly to produce 1 g (44 %) of a tan solid that was carried on without purification. ^{1}H NMR (500 MHz, DMSO-d_6): NMR δ 8.08 (1 H, d, J = 8.9 Hz), 7.12 (1 H, t, J = 7.9), 7.08 (1 H, d, J = 2.5 Hz), 7.94 (2 H, dd, J = 2.6, 8.8 Hz), 6.83 – 6.74 (2 H, m) 6.68 (1 H, dd, J = 2, 7.8 Hz), 4.89 (2 H, s), 3.88 (3 H, s), 3.08, 3.01 (4 H, 2 t, J = 7.8 Hz).

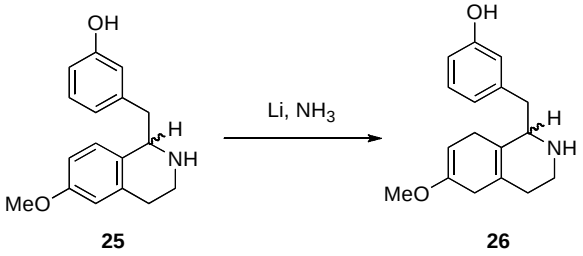
Amine (±)-25[33]:



24 **25**

A stirred solution if 1 g (2.9 mmol) of **24**·HBr in 8.2 ml EtOH was treated with 0.16 g (4.3 mmol) $NaBH_4$ in small portions during 20 min while at 0 – 5 ˚C. After the addition of $NaBH_4$ was complete, the mixture was stirred for 15 min at room temperature, then cooled to 0 - 5 ˚C, and the excess $NaBH_4$ was destroyed with 30 % AcOH to give a final pH of 5. After evaporation, the residue was dissolved in H_2O, rendered alkaline with con. NH_3, and extracted with 2:1 $CHCl_3$/2-propanol (3 x 5 ml). The organic layers were combined, washed with brine, dried over Mg_2SO_4, and evaporated to an off-white solid, which was recrystallized from MeOH to produce 0.28 g (37 %) of **(±)-25** as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): NMR δ 9.25 (1 H, s), 7.14 (1 H, d, J = 8.6 Hz), 7.08 (1 H, t, J = 7.7 Hz), 6.70 – 6.68 (3 H, m), 6.65 – 6.57 (2 H, m), 3.97 (1 H, dd, J = 3.8, 9.3 Hz), 3.70 (3 H, s), 3.10 – 3.00 (2 H, m), 2.80 – 2.60 (4 H, m).

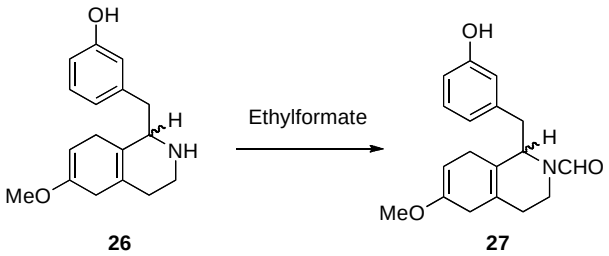
Diene (±)-26[33]:



25 **26**

To 1.85 ml distilled NH_3 were added 26 mg (3.7 mmol) Li metal. To the blue solution was added 1 ml of a 1:1 mixture of dry t-BuOH/THF at -78 ˚C. Into this well stirred mixture a solution of 50 mg (0.19 mmol) of **(±)-25** in 1.5 ml of 1:1 dry t-BuOH/THF was added dropwise during 10 min. The cold bath was switched to a $CHCl_3$/liq N_2 bath and the mixture was allowed to stir at -55 - -65 ˚C for 1 h. The cold bath was dropped, and the reaction was allowed to warm to room temperature. The white residue was diluted with water and extracted with 3:1 $CHCl_3$/2-propanol. The organic extracts were combined, dried over Mg_2SO_4, and evaporated to 28 mg (56 %) of **(±)-26** as a solid. The solid was one spot by TLC in 90:9:1 $CHCl_3$/MeOH/con. NH_3 (Rf = 0.4, stained in iodine). This solid was carried on without purification. ^{1}H NMR (500 MHz, $CDCl_3$): NMR δ 7.13 (1 H, t, J = 7.8 Hz), 6.66 (2 H, d, J = 7.9 Hz), 6.56 (1 H, s), 4.65 (1 H, broad d, J = 3.4 Hz), 3.55 (3 H, s), 3.47 (1 H broad d, J = 10.4 Hz), 3.08 – 2.97 (3 H, m), 2.81 (1 H, m), 2.75 – 2.57 (3 H, m) 2.03 (2 H, broad dd, J = 17, 60.5 Hz).

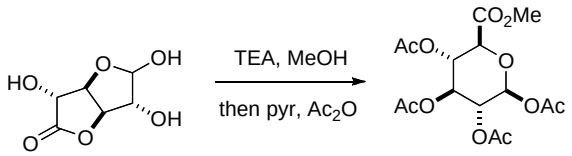
Formamide (±)-27[33]:



26 **27**

18 mg of **(±)-26** in a cold finger was treated with about 2.5 ml of distilled ethylformate. The mixture was allowed to reflux for 12 under an N_2 atmosphere. Evaporation yielded a yellow residue, which was purified by column chromatography in 97:2:1 $CHCl_3$/MeOH/con. NH_3 (Rf = 0.2, stained in CAM) to yield 14 mg (71 %) of **(±)-17** as two rotamers in about a 1:1 ratio apparent by the ^{1}H NMR. ^{1}H NMR (500 MHz, $CDCl_3$): NMR δ 7.91 (1 H, s), 7.37 (1 H, s), 7.24 – 7.07 (3 H, m), 6.67 – 6.54 (6 H, m), 4.80 (1 H, broad t, J = 6.1 Hz), 4.67, 4.66 (2 H, dt, J = 3.4, 14.7 Hz), 4.4 (1 H, q), 3.71 (1 H, d, J = 11.5), 3.56, 3.54 (6 H, 2 s), 3.4 (1 H, m), 3.04 – 2.60 (12 H, m) 2.2 – 1.8 (7 H, m).

Glucopyranuronate (32)[34]**:**

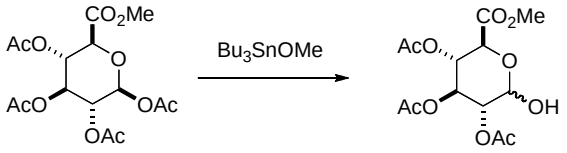


31 **32**

A solution of 8 g (45 mmol) of glucuronolactone **31** and Et_3N (0.8 ml) in MeOH (60 ml) was stirred for 2 h at room temperature then the MeOH was removed by rotoevaporation. The resulting black syrup was dissolved in pyridine (20 ml) and acetic anhydride (30 ml). The mixture was allowed to stir in a cold room between 4 – 5 ˚C for 5 d. The solvent was removed by rotoevaporation, Et_2O was added to the resulting black residue, and the less soluble product was filtered to give a solid (β-isomer **32**), which was recrystallized from 2-propanol to yield 4.69 g (30 %) of **32** as a white solid. The TLC of the recrystallized material was one spot when developed in 2:1 Hex/EtOAc (Rf = 0.29, stained in CAM). [The TLC of the mother liquor showed two closely running spots in 2:1 Hex/EtOAc, presence of the desired β-isomer, **32** (Rf = 0.29), and the α-isomer (Rf = 0.24).] ^{1}H NMR (500 MHz, $CDCl_3$): NMR δ 5.74 (1 H, d, J = 7.8 Hz), 5.29 (1 H, t, J = 9.2 Hz), 5.22 (1 H, t, J = 9.5 Hz), 5.13 (1 H, dd, J = 7.8, 9 Hz), 4.176(1 H, d, J = 9.6 Hz), 3.73 (3 H, s), 2.10 (3 H, s), 2.02 (6 H, s), 2.01 (3 H, s).

[34] Nakajima, R., Ono, M., Aiso, S., Akita, H. *Chem. Pharm. Bull.* **2005**, *53*, 684 – 687.

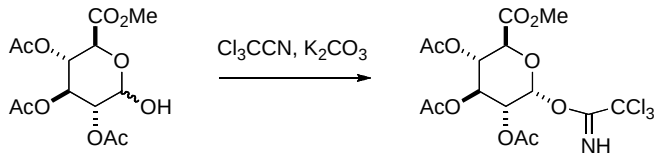
Glucopyranuronate (39)[34]:



32 **39**

A mixture of 2.95 g (7.9 mmol) of 32 and 2.4 ml (7.9 mmol) of tributyltin methoxide in 45 ml dichloroethane was stirred for 5 h at 90 ˚C, and the whole mixture was evaporated to give a crude residue which was purified by column chromatograpy in 3:1 Hex/EtOAc (Rf = 0.17, stained in CAM) to give 1.59 g (61 %) of **39** as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): NMR δ 5.91 (1 H, t, J = 1.5 Hz), 4.94 (1 H, m), 4.78 (1 H, dd, J = 1, 17 Hz), 4.59 (1 H, t, J = 2 Hz), 2.17 (6 H, s), 2.09 (3 H, s).

Trichloroacetimidate donor (33)[34]:

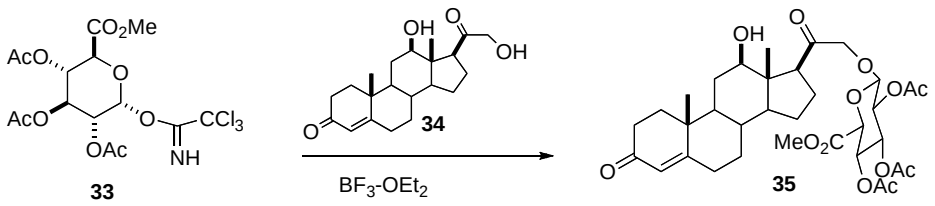


39 **33**

A mixture of 2.34 g (7.0 mmol) of **39**, 1.55 g of K_2CO_3, and 4 Å molecular sieves in 15.6 ml of CH_2Cl_2 was stirred for 25 min at 0 ˚C under an N_2 atmosphere. A solution of trichloroacetonitrile in 7.8 ml of CH_2Cl_2 was added to the reaction mixture, and this mixture was stirred for 2 h at 0 ˚C. The suspension was filtered through celite, and the filtrate was washed

with 7 % $NaHCO_3$ (3 x 5 ml), dried over Mg_2SO_4, and concentrated to a foam. The crude foam was purified by column chromatography in 3:1 Hex/EtOAc (Rf = 0.3, stained in CAM) to isolate 1.88 g (60 %) of solid **33**. ^{1}H NMR (500 MHz, $CDCl_3$): NMR δ 8.71 (1 H, s), 6.62 (1 H, d, J = 3.6 Hz), 5.61 (1 H, t, J = 10 Hz), 5.25 (1 H, dd, J = 9.6, 10.2 Hz), 5.13 (1 H, dd, J = 3.6, 10 Hz), 4.48 (1 H, d, J = 10 Hz) 3.73 (3 H, s), 2.04 (6 H, s), 1.99 (3 H, s).

Protected Corticosterone-glucuronide (35):

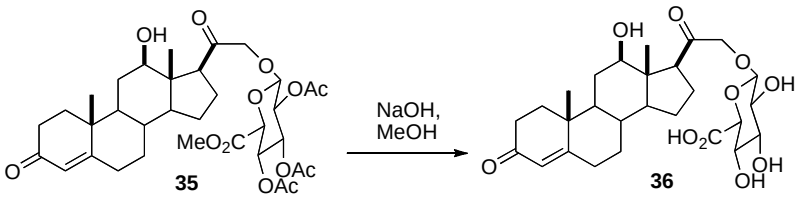


A mixture of 0.83 g (1.7 mmol) of **33**, 0.30 (0.87 mmol) of corticosterone and 4 Å molecular sieves was pumped/purged with N_2/vac, then 17 ml of CH_2Cl_2 was added to the mixture. The mixture was stirred at room temperature for 10 min then placed on an ice bath. 0.11 ml (0.87 mmol) of $BF_3{\cdot}OEt_2$ was added to the mixture at 0 ˚C, then the reaction was stirred at room temperature for 5 h. The reaction was filtered, diluted with hexanes, and washed with saturated $NaHCO_3$ (3 x 3 ml). The organic portion was dried over Mg_2SO_4 and evaporated to a tan foam which was purified by column chromatography in 1:1 Hex/EtOAc (Rf = 0.28, stained in CAM) to isolate compound **35** as a white solid. The NMR data of **35** matched that reported by Ciuffreda et al.[35] with one discrepancy in the ^{13}C data. Ciuffreda at al. reported a resonance at

[35] Ciuffreda, P., Casati, S., De Mieri, M., Ferraboshi, P. *Steroids*, **2009**, *74*, 870 – 875.

54.70 ppm, whereas we observerved this resonance at 57.68 ppm. ^{1}H NMR (500 MHz, $CDCl_3$): NMR δ 5.65 (1 H, d, J = 10 Hz), 5.22 (2 H, dt, J = 30.4 Hz), 5.03 (1 H, dd, J = 7.7, 9.2 Hz), 4.74 (1 H, d, J = 7.7 Hz), 4.44 (1, m), 4.35 (2 H, d, J = 17.2 Hz), 4.17 (1 H, d, J = 17.2), 4.09 (1 H, q, J = 7 Hz), 3.99 (1 H, J = 9.7 Hz), 3.72 (3 H, s), 2.47 (3 H, m), 2.34 (1 H, dt, J = 4, 16.7 Hz), 2.25 – 2.15 (3 H, m), 2.02 (3 H, s), 2.00 (3 H, s), 1.99 (3 H, s), 1.85 (1 H, td, J = 4.6, 13.6), 1.77 – 1.71 (1 H, m), 1.64 – 1.57 (3 H, m), 1.38 – 1.31 (1 H, m), 1.13 – 1.03 (2 H, m), 0.97 (1 H, dd, J = 2.9 11, Hz). ^{13}C (75 MHz, $CDCl_3$): NMR δ 206.45, 199.52, 171.97, 169.92, 169.53, 169.37, 167.43 122.38, 98.93, 72.54, 72.15, 71.74, 70.72, 69.27, 67.97, 58.9, 57.68, 53.15, 47.84, 43.82, 39.24, 34.89, 33.84, 32.61, 32.01, 31.5, 24.53, 22.08, 20.81, 20.73, 20.58, 20.46, 16.25.

Corticosterone-glucuronide (36)[35]:



0.17 g (0.25 mmol) of 35 in 4.2 ml of MeOH was treated slowly with 8.4 ml of about a 1 % sodium hydroxide solution in MeOH (about 8 equivalents of NaOH) over 10 min. The mixture was allowed to stir at room temperature for 24 h. Dowex 50W-X8 was added to reach pH 3.5 – 4. The suspension was filtered through celite, and the filtrate was concentrated to a foam which was purified by column chromatography in 95:4:1 EtOAc/MeOH/H_2O (Rf = 0.25, stained in CAM) to isolate 12 mg (10 %) of pure **36** as a white solid. The NMR data of **36**

matched closely which those of the reported **36**; however the ^{13}C data reported here are shifted 0.50 ppm upfield of the reported ^{13}C data[35] presumably due to a referencing error in the original paper. Here, the central peak of DMSO-d_6 (2.50 ppm for ^{1}H, and 39.5 ppm for ^{13}C) signals were used as an internal reference standard. ^{1}H NMR (500 MHz, $CDCl_3$): NMR δ 5.56 (1 H, s), 5.21 (1 H, d, J = 4.7 Hz), 5.11 (1 H, d, J = 5 Hz), 4.33 (1 H broad d, J = 1.5 Hz), 4.27 (2 H, dd, J = 4.7, 12.5 Hz), 4.23 – 4.17 (2 H, m), 3.55 (1 H, d, J = 9.7 Hz), 3.28 (1 H, t, J = 9.3 Hz), 3.15 (1 H, td, J = 4.3, 8.8 Hz), 3.04 (1 H, m), 2.68 (1 H, t, J = 9 Hz), 2.44 – 2.35 (2 H, m), 2.20 – 2.02 (5 H, m), 1.92 – 1.52 (5 H, m), 1.36 (3 H, s), 1.25 – 0.89 (5 H, m), 0.79 (3 H, s). ^{13}C (75 MHz, $CDCl_3$): NMR δ 207.27, 198.04, 172.27, 170.47, 121.49, 102.37, 76, 75.46, 73.64, 73.09, 71.48, 66.23, 58.03, 56.88, 55.47, 46.39, 43.35, 38.83, 34.01, 33.46, 32.47, 31.3, 31.09, 24.09, 21.73, 20.34, 15.67.

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